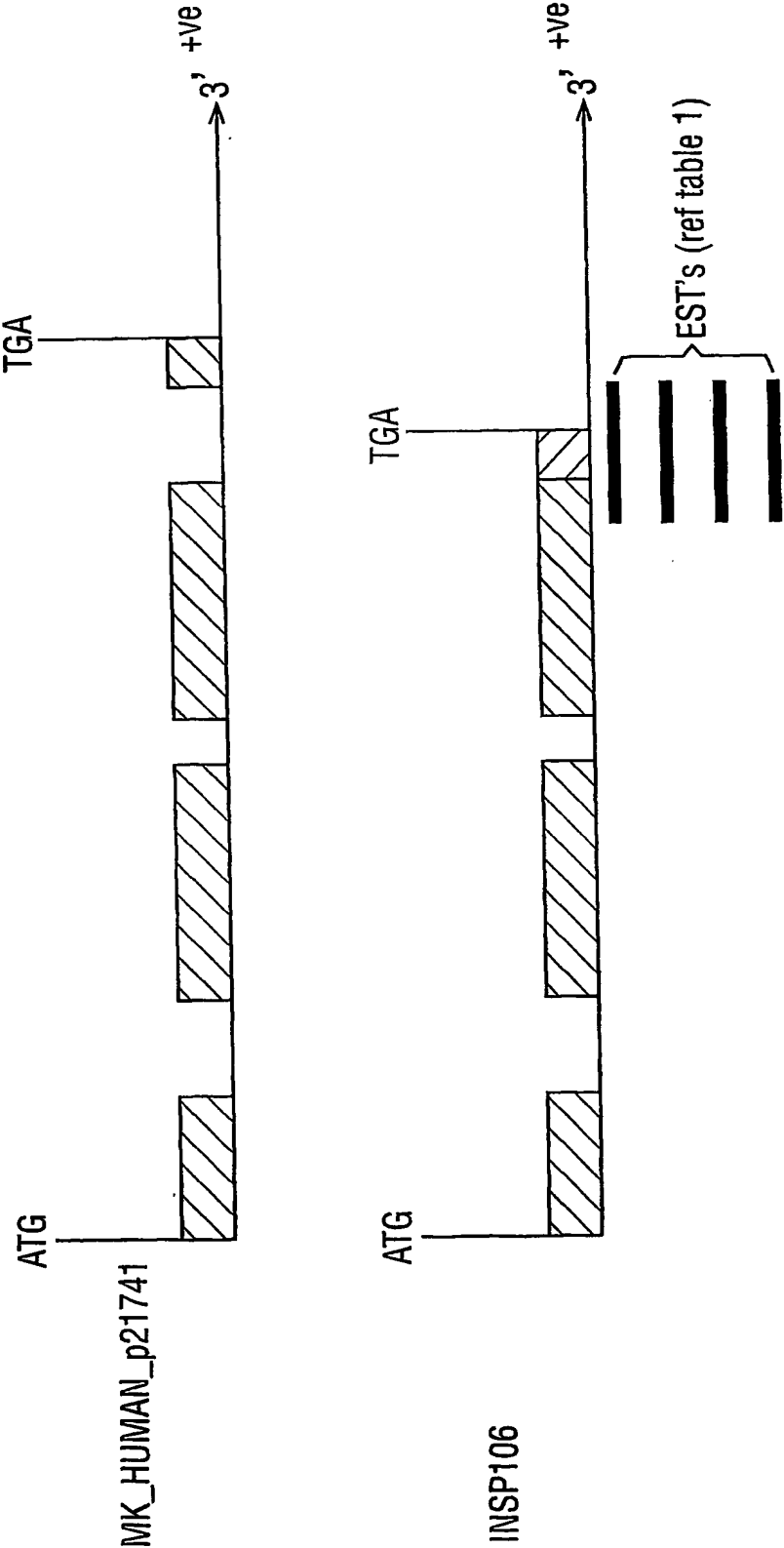


FIG. 1



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FIG. 2(CONTD.)

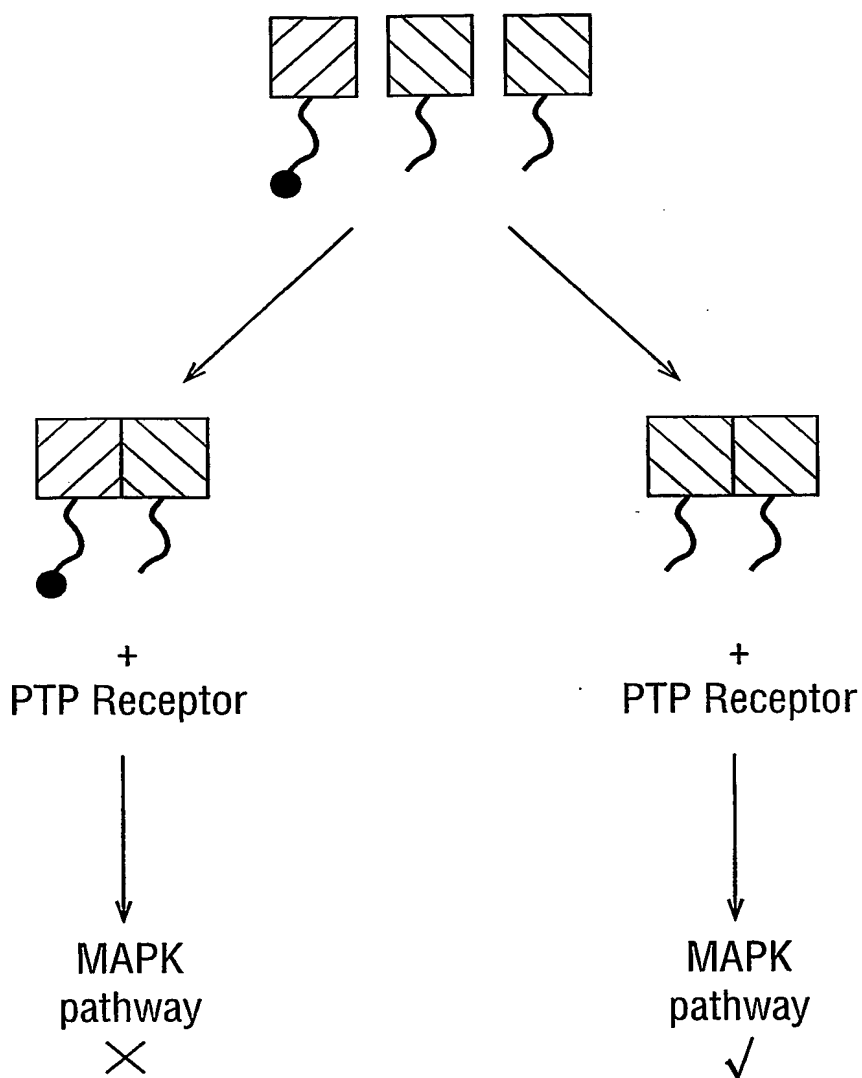
→	swaUP2174PMK_HUMAN	60	KDCGVGF	EG	TCGAQTQRI	C	VP	CNV	DFGADC	YKFE	NWG	ACDGGTGT	V
→	swlclul		KDCGVGF	EG	TCGAQTQRI	C	VP	CNV	DFGADC	YKFE	NWG	ACDGGTGT	V
	swaUPQ9RDEQ/Q9RDE6		KDCGVGF	EG	TCGAQTQRI	C	VP	CNV	DFGADC	YKFE	NWG	ACDGGTGT	V
	swaUP1202PMK_MOUSE		KDCGMGF	EG	TCGAQTQRI	C	VP	CNV	DFGADC	YKFE	NWG	ACDGGTGT	V
	swaUPQ9R1S9/Q9R1S9		KDCGMGF	EG	TCGAQTQRI	C	VP	CNV	DFGADC	YKFE	NWG	ACDGGTGT	V
	swaUP2402PMK_CHICK		KDCGLGY	EG	TCGAQTQRI	C	VP	CNV	DFGADC	YKFE	NWG	ACDGGTGT	V
	swaUP48330PTA1_XENLA		KDCGAGT	EG	TCGAQTQRI	C	VP	CNV	DFGADC	YKFE	NWG	ACDGGTGT	V
	swaUP48331PTA2_XENLA		KDCGAGT	EG	TCGAQTQRI	C	VP	CNV	DFGADC	YKFE	NWG	ACDGGTGT	V
	swaUPQ9W768/Q9W768		GDCGNGI	EA	TCGAQTQRI	C	VP	CNV	DFGADC	YKFE	NWG	ACDGGTGT	V
	swaUPQ9DDG2/Q9DDG2		GDCGNGI	EA	TCGAQTQRI	C	VP	CNV	DFGADC	YKFE	NWG	ACDGGTGT	V
	swaUPQ9W767/Q9W767		GDCGQGM	EG	TCGAQTQRI	C	VP	CNV	DFGADC	YKFE	NWG	ACDGGTGT	V
	swaUP7928/UP7928		GDCGLGT	EG	TCGAQTQRI	C	VP	CNV	DFGADC	YKFE	NWG	ACDGGTGT	V
	swaUP2178PTN_BOVIN		GDCGLGT	EG	TCGAQTQRI	C	VP	CNV	DFGADC	YKFE	NWG	ACDGGTGT	V
	swaUP21240PTN_HUMAN		GDCGLGT	EG	TCGAQTQRI	C	VP	CNV	DFGADC	YKFE	NWG	ACDGGTGT	V
	swaUP20035PTN_MOUSE		GDCGLGT	EG	TCGAQTQRI	C	VP	CNV	DFGADC	YKFE	NWG	ACDGGTGT	V
	swaUPQ9CSX6/Q9CSX6		GDCGLGT	EG	TCGAQTQRI	C	VP	CNV	DFGADC	YKFE	NWG	ACDGGTGT	V
	swaUPQ9CYE3/Q9CYE3		GDCGLGT	EG	TCGAQTQRI	C	VP	CNV	DFGADC	YKFE	NWG	ACDGGTGT	V
	swaUP48332PTB1_XENLA		GDCGLGT	EG	TCGAQTQRI	C	VP	CNV	DFGADC	YKFE	NWG	ACDGGTGT	V
	swaUP48333PTB2_XENLA		GDCGLGT	EG	TCGAQTQRI	C	VP	CNV	DFGADC	YKFE	NWG	ACDGGTGT	V

FIG. 2(CONTD.)


→ swaUP21741MK_HUMAN	QGT L	AR YNAQCQET	RVT	PCTPKT	AAAKAK	GGKD	170
→ swl ch11	QGT L	AR YNAQCQET	RVT	PCTPKT	AAAKQKKE	GGVGLSRGAAPPRL	
swaUPQ9W0E9Q9W0E6	QGT L	AR YNAQCQET	RVT	PCS PKT	AAAKAK	G EK D	
swaUP12025MK_MOUSE	QGT L	AR YNAQCQET	RVT	PCTSKT	AAAKAK	G EK D	
swaUPQ9R1S91Q9R1S9	QGT L	AR YNAQCQET	RVT	PCTSKT	AAAKAK	G EK D	
swaUP24052MK_CHICK	SGT L	AL YNAECCEV	YVY	PCTAKM	AAAKAK	G EK D	
swaUP48330PTA1_XENLA	SGT L	AL YNAECQQT	VEAT	PCS LKT	SSK GX	G EK D	
swaUP48331PTA2_XENLA	SGT L	AL YNAECQQT	VEAA	PCS LKT	SSK GX	G EK D	
swaUPQ9W7681Q9W768	SGT L	AL FNAECQTT	KVS	PCTPKT	PXGGEXK	G EK D	
swaUPQ9D0G21Q9D0G2	SGT L	AL FNAECQTT	KVS	PCTPKT	PXGGEXK	G EK D	
swaUPQ9W7671Q9W767	TGT L	AL FNVHCQQT	VSV	PCTTKV	PKG	GKGN	
swaUP79281PT9281	TGS L	AL HNADCQKT	VTT	PCGKVT	PQAESKK	EGKKQEKMLD	
swaUP21782PTN_BOVIN	TGS L	AL HNADCQKT	VTT	PCGKLT	PQAESKK	EGKKQEKMLD	
swaUP21246PTN_HUMAN	TGS L	AL HNADCQKT	VTT	PCGKLT	PQAESKK	EGKKQEKMLD	
swaUP20935PTN_MOUSE	TGS L	AL HNADCQKT	VTT	PCGKLT	PQAESKK	EGKKQEKMLD	
swaUPQ9CSX61Q9CSX6	TGS L	AL HNADCQKT	VTT	PCGKLT	PQAESKK	EGKKQ	
swaUPQ9CYE51Q9CYE5	TGS L	AL HNADCQKT	VTT	PCGKLT	PQAESKK	EGKKQ	
swaUP48332PTB1_XENLA	SGN L	AL HNADCQKT	VTT	PCGKLT	PQAESKK	EGKKQ	
swaUP48333PTB2_XENLA	NGN L	AL HNADCQKT	VTT	PCGKLT	PQAESKK	EGKKQ	


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FIG. 3



KEY

 Midkine monomer with lysine rich C-terminal tail

 INSP106 with modified C-terminal

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FIG. 4

	10	20	30	40	50	60			
swall	MQHRGFL	LLTLLALL	TSVAKKK	DKVKKGG	PGSECAE	WAWG	PCTPSS	KDCG	VGFREGT
chr11_	MQHRGFL	LLTLLALL	TSVAKKK	DKVKKGG	PGSECAE	WAWG	PCTPSS	KDCG	VGFREGT
	10	20	30	40	50	60			
swall	CGAQTQ	RIRCRV	PCNWK	KEFGAD	CKYKFEN	WGACD	GGTG	TKVRQ	GT
chr11_	CGAQTQ	RIRCRV	PCNWK	KEFGAD	CKYKFEN	WGACD	GGTG	TKVRQ	GT
	70	80	90	100	110	120			
swall	RVTKPCT	PKTKAKA	KA-KKG	KG-----	-----	-----	-----	-----	-----
chr11_	RVTKPCT	PKTKAKA	KA-KKG	KG-----	-----	-----	-----	-----	-----
	130	140	150						

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FIG. 5

1 cgggtgcgtcc gggctagcgg cgagggggccg ccccaagtct tcccaccgcc gccaccttag
 61 cagcccgaact tggggcctgg aaagtggagc acgcggaggt gggagggccc tgcacgcggc
 121 ccccggtggg gaaggggacg ggccagggat tcagactcgg gctctcccct caggatgcag
m q

 181 caccgaggct tcctcctcct caccctcctc gccctgctgg cgetcacctc cgcggtcggc
h r g f l l l t l l a l l a l t s a v a
→
INSP10A-F1
 241 aaaaagaaag ataaggtgaa gaagggcggc ccggggagcg agtgcgctga gtgggcctgg
k k k d k v k k g g p g s e c a e w a w
 301 gggccctgca ccccagcag caaggattgc ggcgtgggtt tccgcgaggg cacctgcggg
g p c t p s s k d c g v g f r e g t c g
 361 gccagaccc agcgcatecc gtgcagggtg ccctgcaact ggaagaagga gtttgagacc
a q t q r i r c r v p c n w k k e f g a
 421 gactgcaagt acaagtttga gaactgggtt gcgtgtgatg ggggcacagg caccaaagtc
d c k y k f e n w g a c d g g t g t k v
 481 cgccaaggca ccctgaagaa ggcgcgtac aatgctcagt gccaggagac catccgcgtc
r q g t l k k a r y n a q c q e t i r v
 541 accaagccct gcaccccaa gaccaaagca aaggccaaag gtcagcgaaa ggagaagggg
t k p c t p k t k a k a k g q r k e k g
 601 gtggggctgt cgcggggggc tgccccccc ccccccgcc tgtgagggga caattccaag
v g l s r g a a p p p p r l
 661 ttaaacctta agttttgagt cctggccagt ggcttcctga catgcctca ctggtctcc
←
INSP10A-R1
 721 ctgcctggaa aagtctgaag atgggcaact caagagaggc cgcaggtgat gctggggaca
 781 taaatcctcc ctggcccaa tagggaccaa ctcaaactac tccattggag catctggctt
 841 aggac

Position and sense of PCR primers →
In italics: predicted signal peptide

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FIG. 7

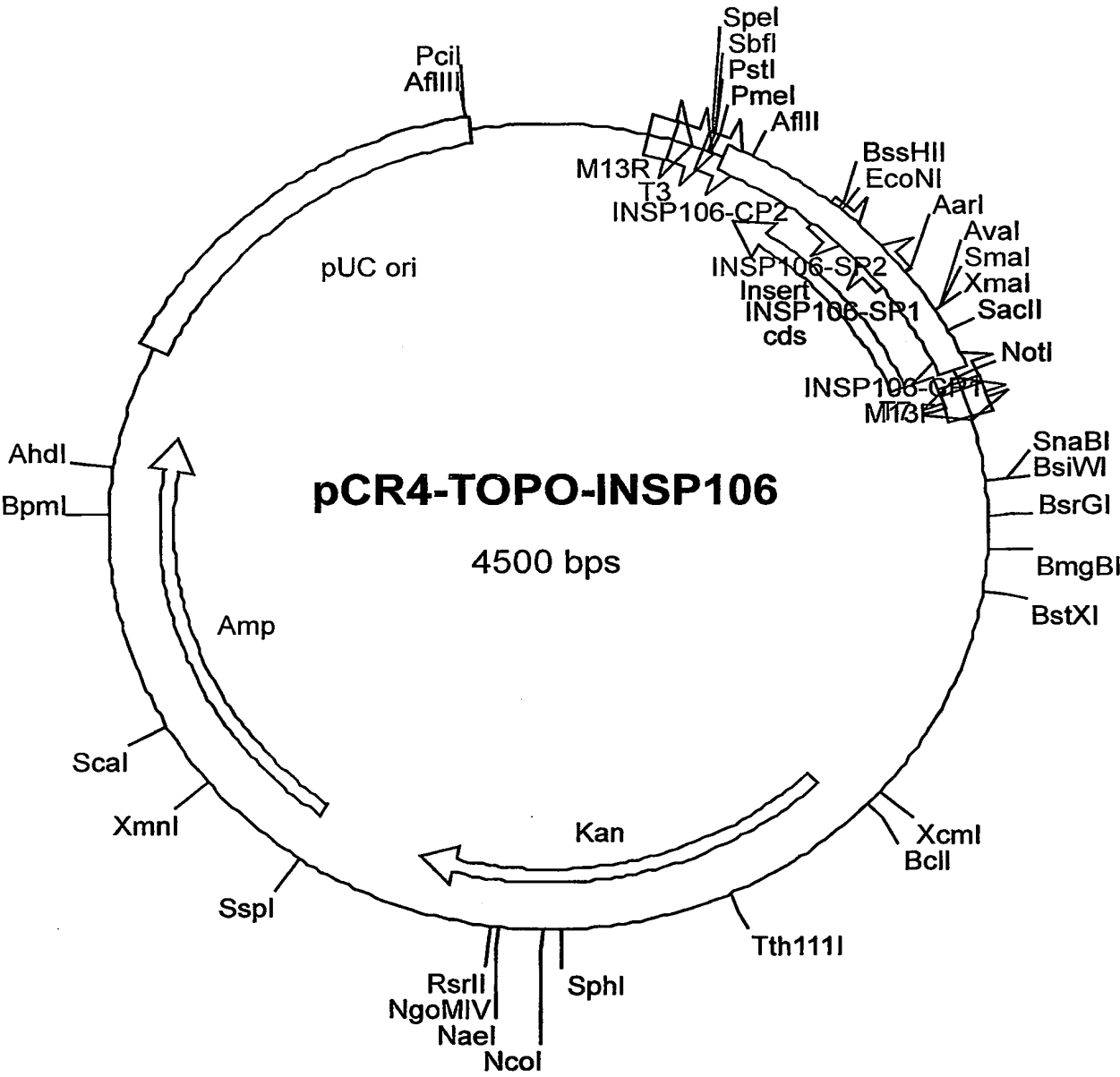
Map of pCR4-TOPO-INSP106

Molecule: pCR4-TOPO-INSP106, 4500 bps DNA Circular

Type	Start	End	Name	Description
MARKER	205		M13R	M13 rev priming site
MARKER	243		T3	T3 priming site
MARKER	295		INSP106-CP2	INSP106-CP2 primer site
MARKER	522		INSP106-SP2	INSP106-SP2 sequencing primer site
MARKER	616		C INSP106-SP1	INSP106-SP1 sequencing primer site
GENE	833	366	C cds	INSP106 cds
MARKER	837		C INSP106-CP1	INSP106-CP1 primer site
REGION	837	295	C Insert	INSP106-CP1/-CP2 PCR product
MARKER	890		C T7	T7 priming site
MARKER	898		M13F	M13 for priming site
GENE	1702	2496	Kan	Kanamycin resistance gene ORF
GENE	2700	3560	Amp	Ampicillin resistance gene ORF
REGION	3705	4378	pUC ori	pUC origin

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FIG. 7(contd)



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FIG. 8

Map of pDONR 221

Molecule: pDONR221, 4759 bps DNA Circular
File Name: pDONR221.cm5

Description:

Type	Start	End	Name	Description
REGION	295	268	C rrnB T2	transcription termination sequence
REGION	470	427	C rrnB T1	transcription termination sequence
REGION	536	553	21M13	M13 Forward primer
REGION	570	801	attP1	
GENE	1197	1502	ccdB	
GENE	1844	2503	Cm r	Chloramphenicol resistance gene
REGION	2751	2982	attP2	
REGION	3040	3023	C M13 Rev	M13 Reverse primer
GENE	3153	3962	Kan r	
REGION	4083	4756	pUC ori	

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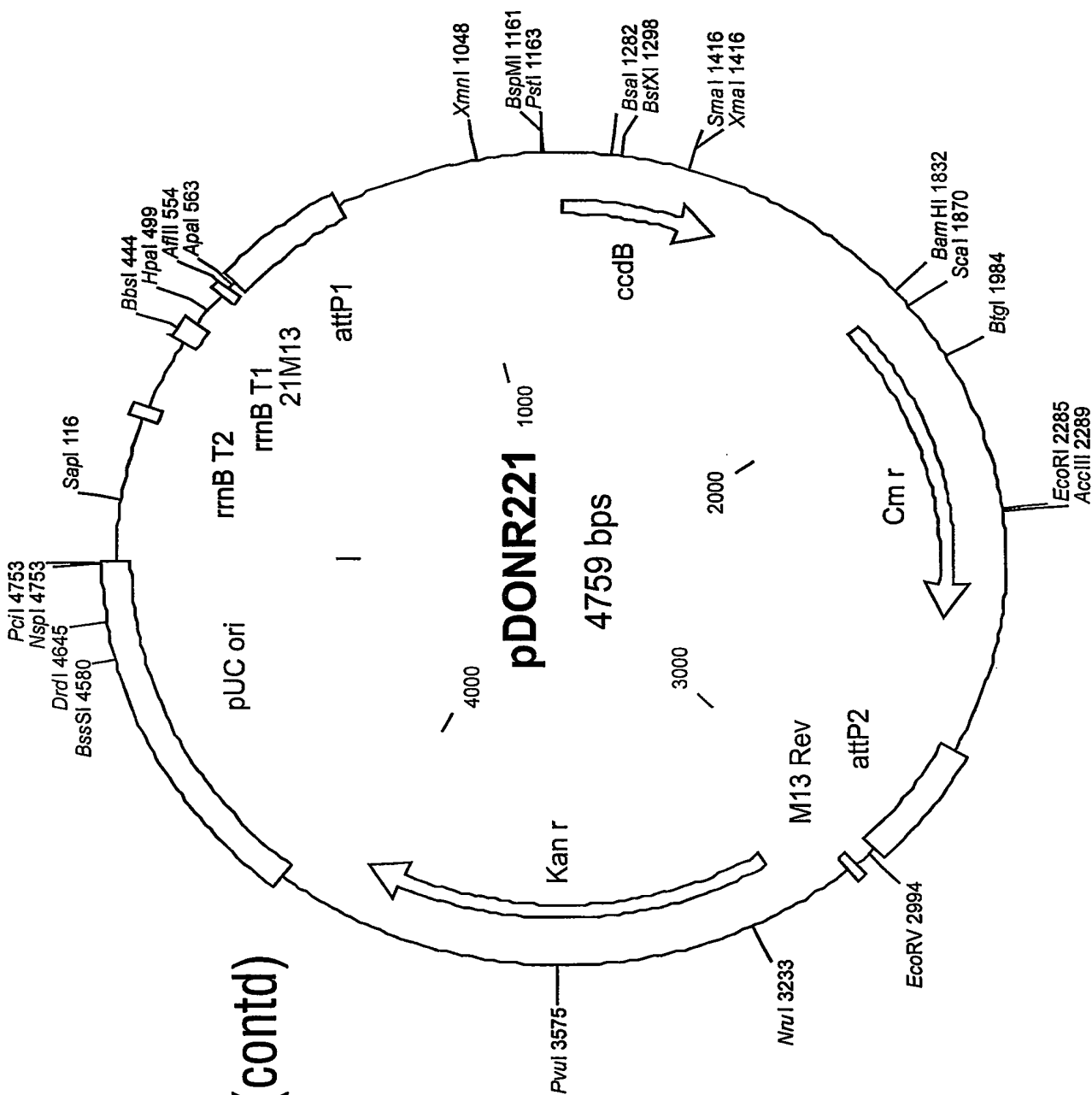


FIG. 8(contd)

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FIG. 9

Map of expression vector pEAK12d

Molecule: pEAK12 d, 8760 bps DNA Circular
 File Name: pEAK12DEST.cm5

Description: Mammalian cell expression vector (plasmid ID 11345)

Molecule Features:

Type	Start	End	Name	Description
REGION	2	595		pmb-ori
GENE	596	1519	Amp	
REGION	1690	2795	EF-1alpha	
REGION	2703	2722		position of pEAK12F primer
REGION	2796	2845		MCS
MARKER	2855		attR1	
GENE	3256	3915	CmR	
GENE	4257	4562	ccdB	
MARKER	4603		C attR2	
REGION	4733	4733		MCS
REGION	4734	5162		poly A/splice
REGION	4819	4848	C	position of pEAK12R primer
GENE	5781	5163	C PUR	PUROMYCIN
REGION	6005	5782	C tK	tK promoter
REGION	6500	6006	C Ori P	
GENE	8552	6500	C EBNA-1	
REGION	8553	8752	sv40	

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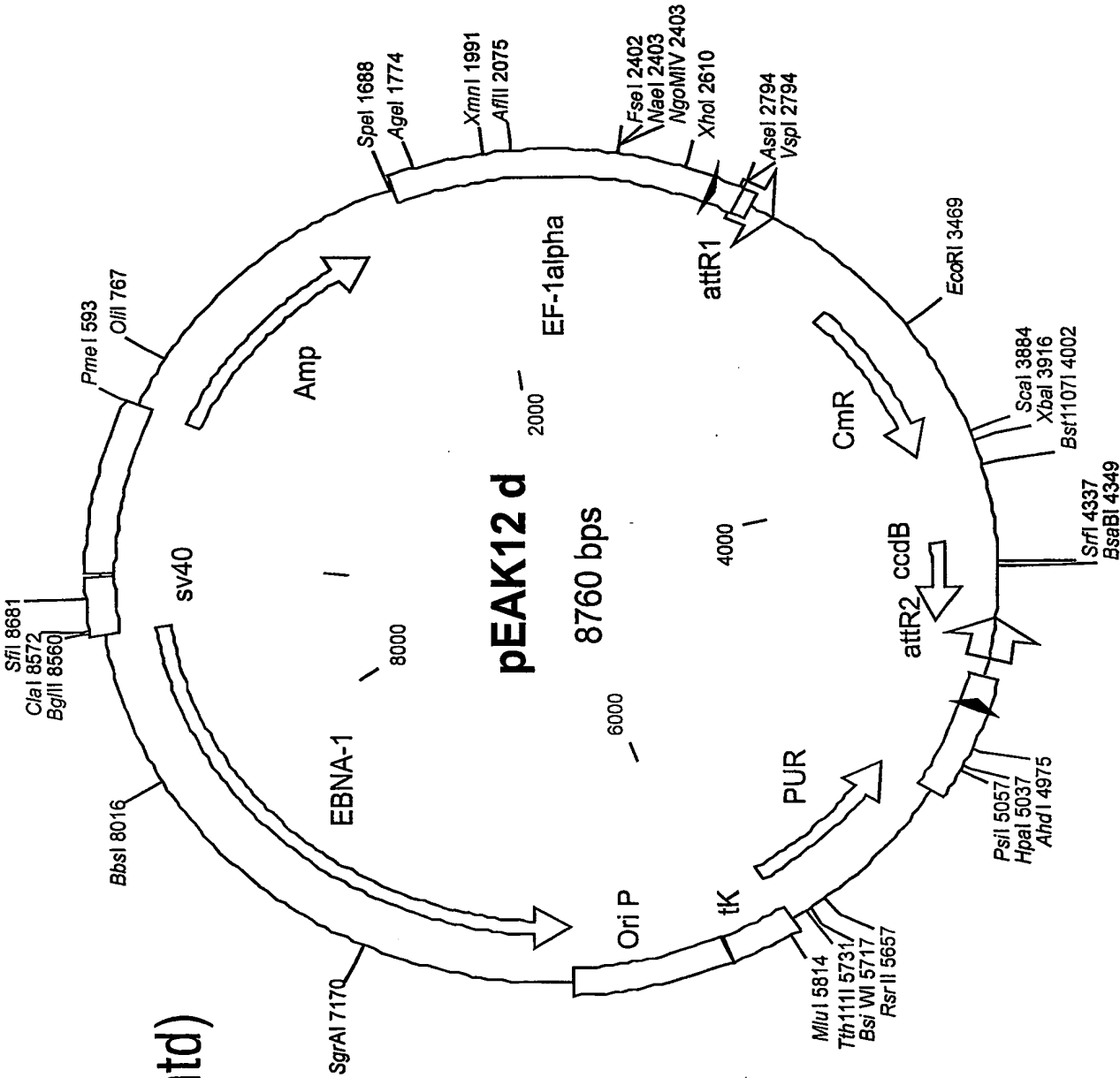


FIG. 9(contd)

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FIG. 10

Map of Expression vector pDEST12.2

Molecule: pDEST 12.2, 7278 bps DNA Circular
 File Name: pDEST12-2.cm5

Description: Eukaryotic expression vector

Type	Start	End	Name	Description
REGION	15	608	CMV	CMV promoter
MARKER	648		M13R	M13R primer
REGION	687	706	SP6	SP6 promoter
REGION	730	854	attR1	
GENE	963	1622	Cm	
GENE	1964	2269	ccdB	
REGION	2310	2434	attR2	
GENE	2484	2464	C T7	T7 promoter
MARKER	2512		C 21M13	21M13 primer
REGION	2784	3050	pA	SV40 polyadenylation signal
REGION	3176	3631	f1	f1 intergenic region
REGION	3791	4099	P SV40	SV40 ori & early promoter
GENE	4158	4952	Neo	
REGION	5016	5064	pA	synthetic poly adenylation signal
GENE	5475	6335	Amp	
REGION	6480	7153	ori	pUC ori

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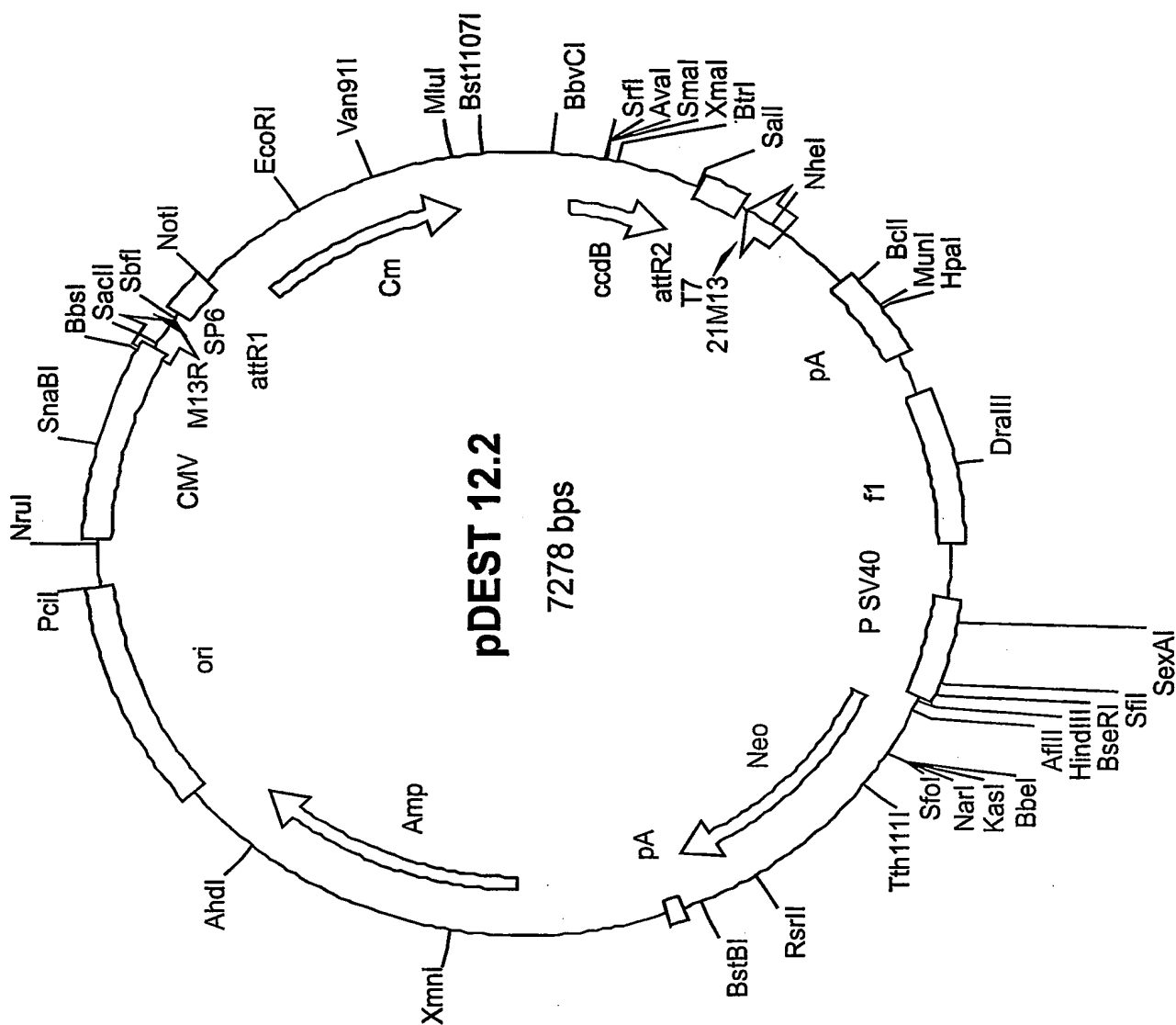


FIG. 10(contd)

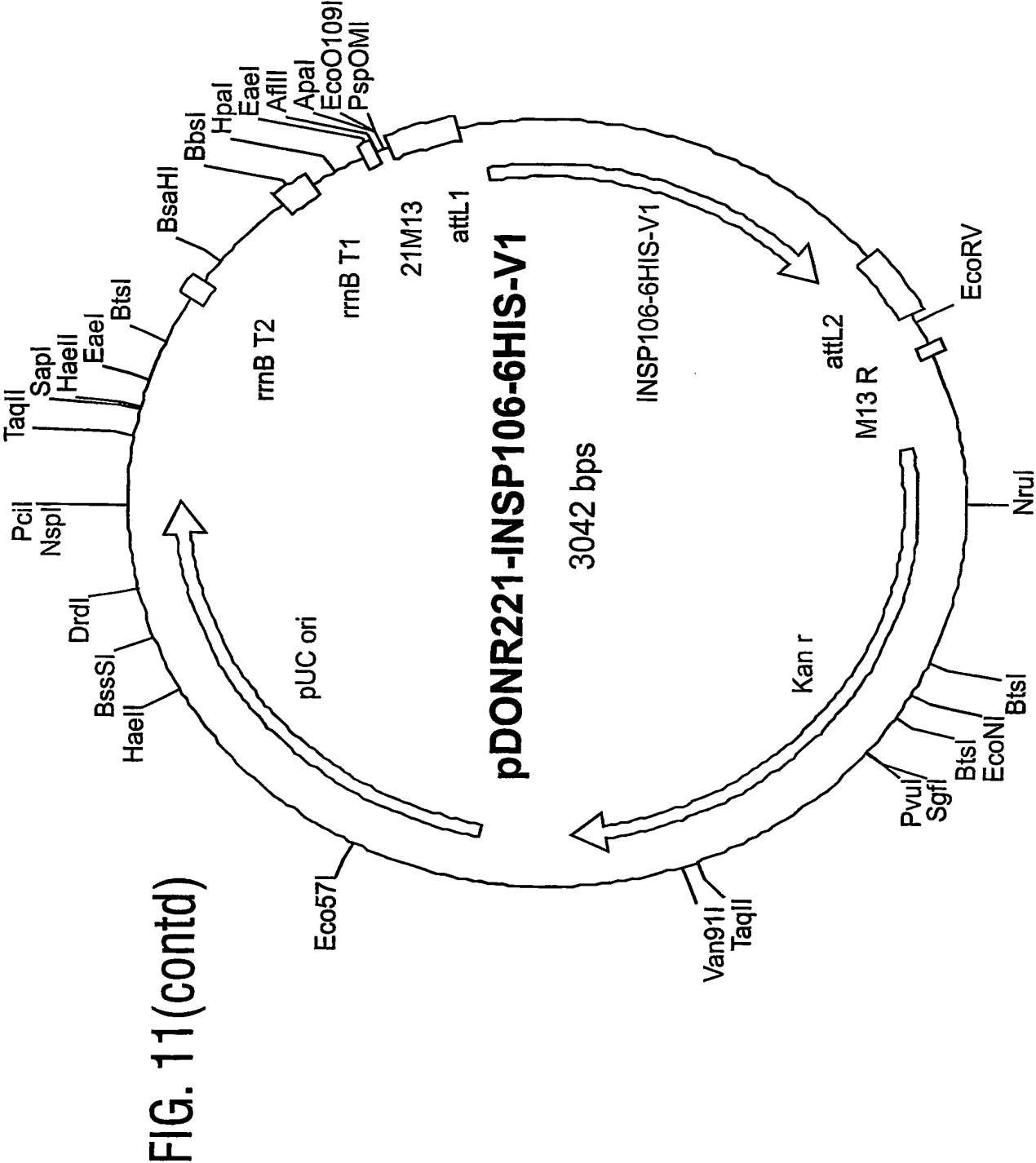
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FIG. 11

Map of pDONR221-INSP106-6HIS

Molecule: pDONR221-INSP106-6HIS-V1, 3042 bps DNA Circular

Type	Start	End	Name	Description
REGION	295	268	C rrnB T2	transcription termination sequence
REGION	470	427	C rrnB T1	transcription termination sequence
REGION	536	553	21M13	21M13 primer
REGION	570	651	attL1	
GENE	677	1162	INSP106-6HIS-V1	
REGION	1177	1265	attL2	
REGION	1323	1307	C M13 R	M13R primer
GENE	1436	2245	Kan r	
GENE	2366	3039	pUC ori	



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FIG. 12

Map of pEAK12d-INSP106-6HIS

Molecule: pEAK12d-INSP106-6HIS-V1, 7435 bps DNA Circular

Type	Start	End	Name	Description
REGION	2	595	pmb-ori	
GENE	596	1519	Amp	
REGION	1690	2795	EF-1alpha	
REGION	2796	2845	MCS''	
REGION	2855	2874	attB1	
GENE	2888	3373	INSP106-6HIS-V1	
REGION	3381	3402	attB2	
REGION	3408	3408	'MCS	
REGION	3409	3837	'A	poly A/splice
GENE	4456	3838	C PUR	PUROMYCIN
REGION	4680	4457	C tK	tK promoter
REGION	5175	4681	C Ori P	
GENE	7227	5175	C EBNA-1	
REGION	7228	7427	sv40	

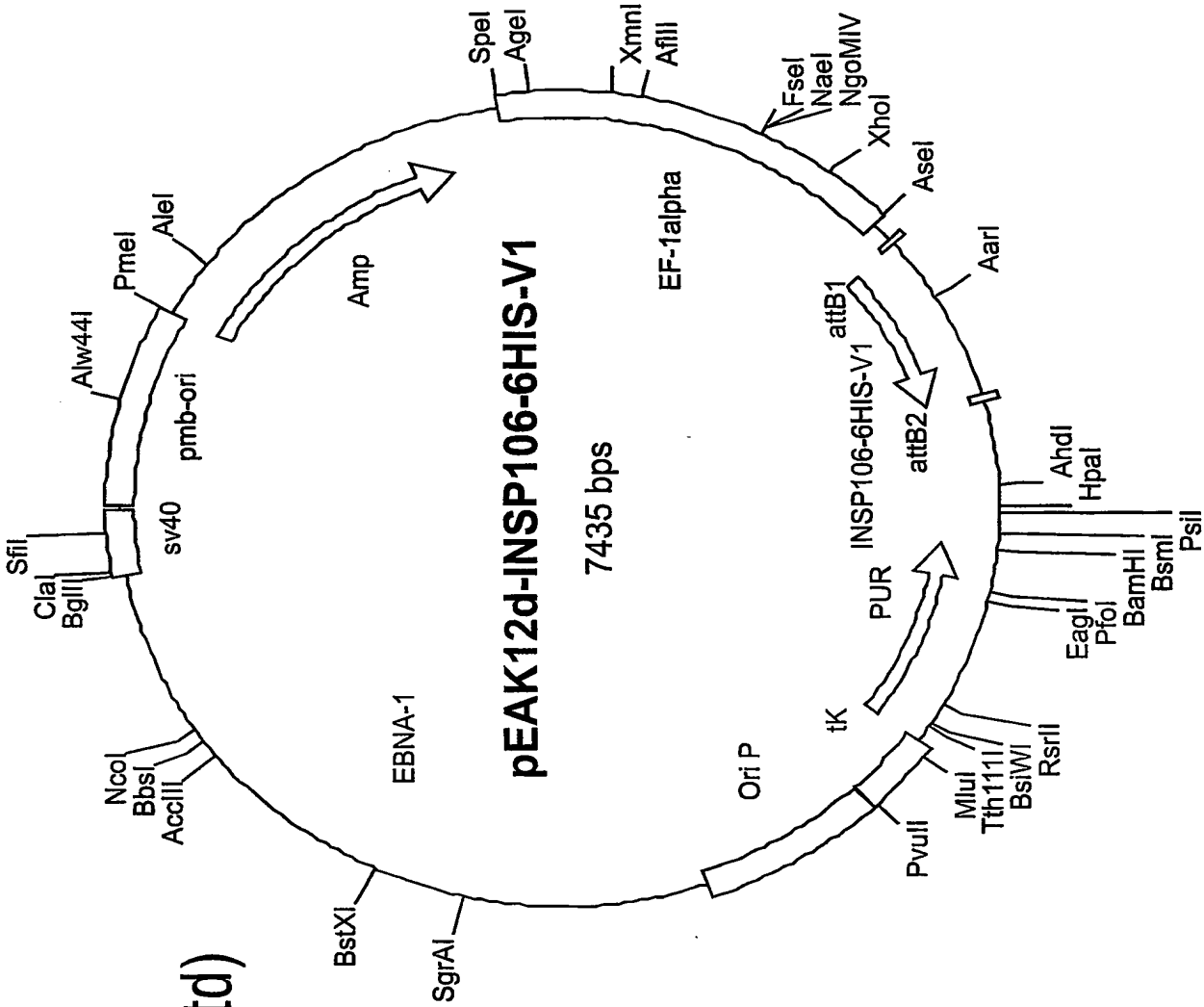


FIG. 12(contd)

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FIG. 13

Map of pDEST12.2-INSP106-6HIS

Molecule: pDEST12.2-INSP106-6HIS-V1, 6121 bps DNA Circular

Type	Start	End	Name	Description
GENE	15	537	CMV promoter	
REGION	648	665	M13rev	M13R primer
REGION	687	704	SP6	SP6 primer
REGION	730	762	attB1	
GENE	763	1248	INSP106-6HIS-V1	
REGION	1252	1277	attB2	
REGION	1327	1307	C T7	T7 promoter
REGION	1356	1338	C 21M13	21M13 primer
GENE	1462	1824	pA	SV40 polyadenylation signal
GENE	2018	2474	f1	f1 intergenic region
GENE	2538	2956	P SV40	SV40 ori & early promoter
GENE	3001	3795	Neo	
GENE	3859	3907	pA	poly adenylation signal
GENE	4318	5178	Amp	
GENE	5327	5966	ori	pUC ori

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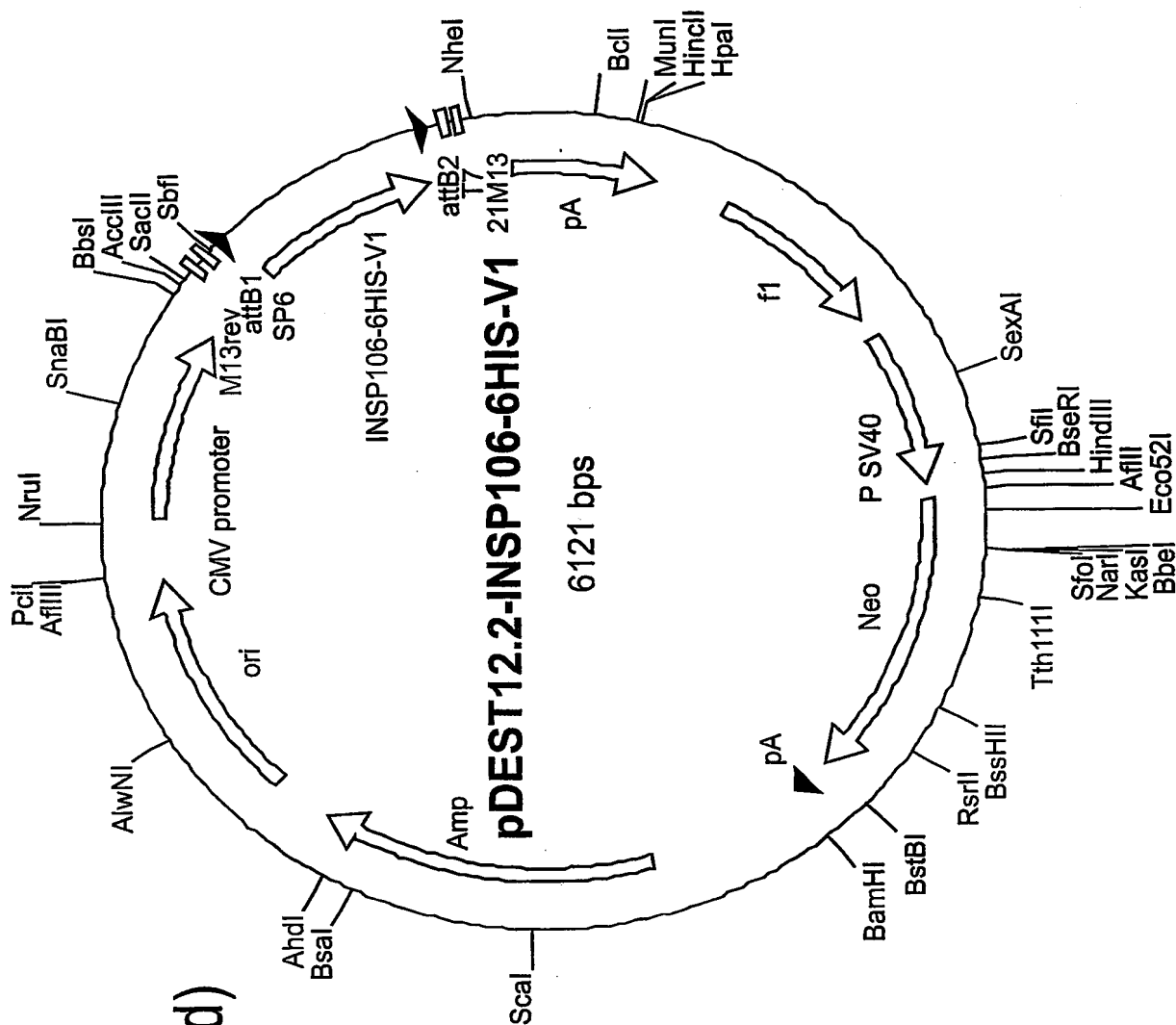


FIG. 13(contd)